UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
CHEMICAL SAFETY AND
POLLUTION PREVENTION

July 6, 2010

MEMORANDUM

Subject: Acute Toxicity Review for EPA Reg. No. 67071-LI
DP Barcode: D368349

From: Chris Jiang, Chemist
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Product Science Branch
Antimicrobials Division (7510P)

Chris Jiang

Through: Karen Hicks, Team Leader
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Chris Jiang for KEH 7/6/10

To: Marshall Swindell PM 33\Abigail Downs
Regulatory Management Branch I
Antimicrobials Division (7510P)

Applicant: Thor GmbH

FORMULATION FROM LABEL:

Active Ingredient(s):	% by wt.
4,5-Dichloro-2-n-octyl-3(2H)-isothiazolone	97.10 %
Other Ingredient(s):	2.90 %
Total:	100.00 %

BACKGROUND: The registrant has submitted an acute toxicity package for the registration of this industrial microbiocide. The package includes a label, a Confidential Statement of Formula, a summary of acute toxicity characteristics (MRID 47802210), an acute oral toxicity study (MRID 47802211), an acute dermal toxicity study (MRID 47802212), an acute inhalation study (MRID 47802213), a skin irritation study (MRID 47802214), and a dermal sensitization study (MRID 47802215). The registrant is citing the eye irritation for another product to fulfill the guideline for eye irritation. The contractor has conducted a primary review of this submission and Product Science Branch of Antimicrobials Division has conducted a secondary review, which supersedes the primary review.

RECOMMENDATIONS:

1. The studies for acute oral toxicity and acute inhalation are acceptable.

2. The studies for acute dermal toxicity and acute dermal irritation are unacceptable. The studies do not indicate whether the test material was moistened. If the test substance was moistened, the lab report does not indicate the procedure used.

3. Because the test material in the cited study caused severe irritation of the eye, e.g., corneal opacity of grade 3, the acute toxicity category for eye irritation is I. The iris and cornea could not be observed because of extreme swelling of the eyelids.

4. The skin sensitization is upgradable when the registrant submits the age of the guinea pigs. Further, the lab report does not indicate whether the test material was moistened. If the test substance was moistened, the lab report does not indicate the procedure used. If the test substance was not a sensitizer, the study would have been unacceptable.

5. The acute toxicity profile for 67071-LI is currently:

Study	MRID Number	Toxicity Category	Study Status
Acute Oral Toxicity	47802211	III	Acceptable
Acute Dermal Toxicity	47802212		Unacceptable
Acute Inhalation Toxicity	47802213	II	Acceptable
Primary Eye Irritation	00126791	I	Cited
Primary Skin Irritation	47802214		Unacceptable
Dermal Sensitization	47802215	Sensitizer	Upgradable

LABELING

No precautionary labeling or first aid statements can be determined at the present time. The precautionary labeling and first aid statements will be reconsidered when the registrant supplies the requested data.

DATA REVIEW FOR ACUTE ORAL TOXICITY TESTING (81-1, 870.1100)

Product Manager: Marshall Swindell
Reviewer: Chris Jiang
MRID No.: 47802211
Study Completion Date: Dec. 29, 2000
Report No.: 2867
Testing Laboratory: JAI Research Foundation
Author: Mital D. Rana

Quality Assurance (40 CFR 160.12): A statement of GLP compliance was included.

Test Material: Acticide DCOIT, batch LM 437, white\yellow crystalline solid
Dosage: 200, 500, and 2000 mg/kg

Species: Wistar rats (nine males and six females)
Age: Nine to twelve weeks at time of dosing
Weight: ♂: 163 to 259 grams before dosing; ♀: 169 to 195 grams before dosing
Source: Breeding facility, Jai Research Foundation, India

Conclusions:

1. **LD_{50} (mg/kg):** $500 < LC_{50} < 2000$ mg/kg
2. **The estimated LD_{50} is greater than 500 mg/kg and less than 2000 mg/kg.**
3. **Tox. Category**: III **Classification**: Acceptable

Procedure (Deviations from 81-1): The Acute Toxic Class Method was used to evaluate the acute oral toxicity of the test substance. Peanut oil was used instead of water as the vehicle. There were also deviations in husbandry and observation periods; however, these deviations had no impact on the integrity of the study.

Results:

Reported Mortality

Dose Level (mg/kg)	Number Dead / Number Tested		
	Males	Females	Total
200	0 / 3	0 / 3	0 / 6
2,000	3 / 3	0 / 0	3 / 3
500	0 / 3	0 / 3	0 / 6

Observations:
200 mg/kg (3 males and 3 females): No mortality was observed. Clinical signs included lethargy. One male was observed to have a nostril discharge and another male was observed to have abdominal breathing.
2000 mg/kg (3 males): All animals were found dead on day 1. Clinical signs included lethargy, unusual locomotion, toe walking, abdominal breathing, gasping, nostril discharge, diarrhea, piloerection, and salivation.
500 mg/kg (3 males and 3 females): Clinical signs included lethargy, toe walking, abdominal breathing, and piloerection.

Gross Necropsy Findings:

200 mg/kg (3 males and 3 females): Gross necropsies revealed patchy congestion of the kidneys, partially blotched kidneys, hepatisation in the right cranial lobe of the lungs, mottling of the liver, and atrophy of the spleen.
2000 mg/kg (3 males): Gross necropsies showed mottling of the liver, emphysema of the lung, diffused congestion of the lung, mucus exudation of the intestines, and consolidation of the lungs.
500 mg/kg (3 males and 3 females): Gross necropsies of the males were unremarkable. Gross necropsies of the females revealed diffused consolidation of the lungs, atrophy of the spleen, bilateral patchy congestion of the kidneys, diffused emphysema with pinpoint hemorrhages of the lungs, diffused pinpoint hemorrhages of the kidneys, and diffused pinpoint hemorrhages in left lobe of the lung.

DATA REVIEW FOR ACUTE DERMAL TOXICITY TESTING (81-2, 870.1200)

Product Manager: Marshall Swindell
Reviewer: Chris Jiang
MRID No.: 47802212
Study Completion Date: Dec. 29, 2000
Report No.: 2868
Testing Laboratory: JAI Research Foundation
Author: Dr. T. Purshottam, Ph.D., M.Sc.

Quality Assurance (40 CFR 160.12): A statement of GLP compliance was included.

Test Material: Acticide DCOIT, batch LM 437, white\yellow crystalline solid
Dosage: 2000 mg/kg

Species: Wistar rats (ten males and ten females)
Age: Not given
Weight: ♀: 202 to 220 grams before dosing; ♂: 201 to 237 grams before dosing
Source: Breeding facility, Jai Research Foundation, India

Conclusions:

1. **LD_{50} (mg/kg):** **Males** > 2000 mg/kg
   Females > 2000 mg/kg
   Combined > 2000 mg/kg
2. **The estimated LD_{50} is greater than 2000 mg/kg.**
3. **Tox. Category**: Unknown **Classification**: Unacceptable

Procedure (Deviations from 81-2): A range-finding study was undertaken. The ages of the animals were not reported. These deviations had no impact on the integrity of the study. This study does not indicate whether the test material was moistened. If the test substance was moistened, the lab report does not indicate the procedure used.

Results:

Reported Mortality

Dosage (mg/kg)	(Number Deaths/Number Tested)		
	Males	Females	Combined
Control group	0/5	0/5	0/10
2000	0/5	0/5	0/10

Observations: No clinical signs were observed in the control group. Clinical signs in the treated group included abdominal breathing, rough coat, and erythema.

Gross Necropsy Findings: Externally, gross necropsies of the control group were unremarkable. Internally, gross necropsies revealed consolidation of the lungs, diffused pinpoint hemorrhages of the lungs, patchy congestion of the kidneys, diffused pneumonic foci of the lungs, and bilateral hydrometra. Externally, gross

necropsies of the treated group showed epidermal thickening, alopecia, and erythema. Internally, gross necropsies revealed consolidation of the lungs, patchy congestion of the kidneys, diffused pinpoint hemorrhages of the lungs, diffused pneumonic foci of the lungs, and bilateral hydrometra.

DATA REVIEW FOR ACUTE INHALATION TOXICITY (§81-3, 870.1300)

Product Manager: Marshall Swindell
MRID No.: 47802213
Reviewer: Chris Jiang
Study Completion Date: June 1, 2001
Report No.: 2871
Testing Laboratory: JAI Research Foundation
Author: Dr. T. Purshottam, Ph.D., M.Sc.

Quality Assurance (40 CFR 160.12): A statement of GLP compliance was included.

Test Material: Acticide DCOIT, batch LM 437, white, beige crystals
Dosage: 0.143 mg/L, 0.221 mg/L, and 0.289 mg/L

Species: Wistar rats
Age: Ten weeks
Weight: ♀: 123 g to 191 g before dosing; ♂: 130 g to 181 g before dosing
Source: Breeding facility, Jai Research Foundation, India

Summary:

1. **LC_{50} (mg/L):** 0.164 mg/L (95% C.I.: 0.123 to 0.219 mg/L)
2. **The LC_{50} is 0.164 mg/L.**
3. **MMAD**: 3.43 μ at 0.289 mg/L
   3.44 μ at 0.221 mg/L
   3.37 μ at 0.143 mg/L
4. **Toxicity Category**: II **Classification**: Acceptable

Concentration:

Group	Gravimetric Exposure Concentration (mg/L)	Nominal Concentration (mg/L)
Group I (Control)	--	--
II	0.289	2.0
III	0.221	1.5
IV	0.143	1.0

Results:

Reported Mortality

Treatment	Exposure Concentration (mg/L)	Number Dead / Number Tested		
		Males	Females	Combined
Group I (Control)	dimethyl sulphoxide	0 / 5	0 / 5	0 / 10
Group II	0.289	4 / 5	5 / 5	9 / 10
Group III	0.221	3 / 5	4 / 5	7 / 10
Group IV	0.143	2 / 5	2 / 5	4 / 10

Chamber Atmosphere

Exp. Conc. (mg/L)	Sample (hour)	MMAD (μ)	GSD (μ)	% of Particles							
				Particle Size Range (μ)							
				0 - 0.69	0.69 - 1.03	1.03 - 1.72	1.72 - 2.85	2.85 - 4.45	4.45 - 7.10	7.10 - 11.2	>11.2
0	1	--	--	3.85	3.85	11.54	19.23	34.62	3.85	7.69	15.38
	2			0.00	4.17	12.50	20.83	37.50	4.17	8.33	12.50
	3			4.00	4.00	12.00	20.00	32.00	4.00	8.00	16.00
	4			3.85	3.85	11.54	19.23	34.62	3.85	7.69	15.38
0.289	1	3.43	2.15	0.84	1.35	18.01	19.02	20.20	20.88	11.95	7.74
	2			0.85	1.53	17.86	18.88	20.24	20.58	12.41	7.65
	3			0.84	1.35	18.07	18.92	19.93	20.78	12.67	7.43
	4			0.84	1.35	17.88	19.06	20.07	21.08	11.80	7.93
0.221	1	3.44	2.15	0.66	1.32	18.10	18.98	20.31	20.97	11.92	7.73
	2			0.67	1.57	18.20	19.10	20.00	20.45	12.36	7.64
	3			0.67	1.35	18.20	18.88	19.78	21.35	11.91	7.87
	4			0.66	1.33	18.14	19.03	20.13	20.80	11.95	7.96
0.143	1	3.37	2.18	1.02	1.70	18.03	19.05	20.07	20.75	11.90	7.48
	2			1.04	2.08	17.65	19.38	20.07	20.76	11.76	7.27
	3			1.05	1.74	18.47	17.42	20.56	21.25	12.20	7.32
	4			1.02	2.38	17.69	19.05	20.07	20.41	11.90	7.48

Chamber Environment During Exposure

Chamber Volume (L)	63.5 (total capacity)
Average Total Airflow Volume (Lpm)	10
Air Changes Per Hour	Not reported
Mean Oxygen Content (%)	20.2
Mean Temperature (°C)	21.7
Mean Relative Humidity (%)	43.8

Clinical Observations:

Control: No mortality was observed in the control group.

0.289 mg/L: 90 percent mortality was observed. Four males and five females died by Day 4 of the experiment. 10 percent mortality occurred at the 4th hour after exposure and 40, 10, and 30 percent mortality was observed at the Day 1, Day 2, and Day 3 observations, respectively. All animals exhibited symptoms of abdominal breathing from the 2nd to 4th hour after exposure. Nasal irritation was observed in all ten animals at the 3rd hour after exposure and in the nine surviving animals at the 4th hour after exposure. Four males showed symptoms of gasping at the Day 1 observation, with two male animals showing symptoms of gasping at the Day 2 observation. Three males showed abdominal breathing at the Day 1 observation. One male (12M) was lethargic at the Day 3 and Day 4 observation. The last two surviving female animals (16F and 19F) showed symptoms of lethargy and gasping at the Day 1 and Day 2 observation.

0.221 mg/L: 70 percent mortality was observed. Three boars and four sows died by Day 4 of the experiment. 50 and 20 percent mortality occurred at the Day 1 and Day 3 observations, respectively. Two males and one female survived. All ten animals exhibited symptoms of abdominal breathing and nasal irritation from the 2nd to 4th hour after exposure. Tremors were observed in all ten animals at the 4th hour after exposure. The two

surviving male animals (22M and 23M) showed symptoms of gasping at the Day 1, Day 2, and Day 3 observation and appeared normal over the remainder of the 14-day experiment. Three sows (27F, 28F, and 29F) showed symptoms of gasping at the Day 1 and Day 2 observation periods. The last surviving female animal (28F) exhibited symptoms of gasping at the Day 3 observation.

0.143 mg/L: 40 percent mortality was observed. Two boars and two sows died by Day 2 of the experiment. Three males and three females survived. All ten animals showed symptoms of nasal irritation from the 2nd to 4th hour after exposure, with abdominal breathing from the 2nd to 3rd hour after exposure. Gasping was recorded for all ten animals at the 4th hour after exposure. The six surviving animals (31M, 33M, 35M, 36F, 37F, and 40F) showed symptoms of gasping at the Day 1 and Day 2 observations, with lethargy at the Day 2 observation. Three of the six surviving animals (31M, 33M, and 35M) showed symptoms of lethargy at the Day 3 observation period.

Gross Necropsy Findings:
Gross necropsy showed that the lesions in different treatment groups were not consistent in nature and appear to be of the incidental type. The findings did not seem to be dose related.

Control: External examination was unremarkable. Internal examination revealed lesions in the lungs (i.e., emphysema, hepatisation, congestion) of three animals and discoloration of the left adrenal in one animal.

0.289 mg/L: External examination of the decedents was unremarkable, except for female who showed congestion of the mucous membrane. External examination of the euthanized animals was unremarkable. Internal examination of the decedents revealed lesions in the lungs (i.e., emphysema, hepatisation, congestion), mottling in the liver, blotching in the kidney, congestion in the spleen, and/or hydrometra in the uterus. Internal examination of the euthanized animal revealed lesions in the lungs (i.e., emphysema, congestion).

0.221 mg/L: External examination of the decedents was unremarkable except for congestion of the mucous membrane, nasal discharge, and/or mucous crest formation in two female animals. External examination of the euthanized animals was unremarkable. Internal examination of the decedents revealed lesions in the lungs (i.e., emphysema, congestion, hemorrhage), in the liver (mottling, hepatomegaly, discoloration), blotching in the kidney, congestion and frothy exudation in the trachea, hemorrhage in the adrenals, and/or hemorrhagic contents in the small intestine. Internal examination of the euthanized animals revealed lesions in the lungs (i.e., emphysema, hepatisation, hemorrhage).

0.143 mg/L: External examination of the decedents and euthanized animals was unremarkable. Internal examination of the decedents revealed lesions in the lungs (i.e., emphysema, congestion) and in the liver (mottling). Internal examination of the euthanized animals revealed lesions in the lungs (i.e., emphysema, hepatisation, hemorrhage), and/or hydrometra of the uterus.

DATA REVIEW FOR PRIMARY SKIN IRRITATION TESTING (81-5, 870.2500)

Product Manager: Marshall Swindell
MRID No.: 47802214

Reviewer: Chris Jiang
Study Completion Date: Dec. 29, 2000
Report No.: 2869

Testing Laboratory: JAI Research Foundation
Author: Dr. T. Purshottam, Ph.D., M.Sc.

Quality Assurance (40 CFR 160.12): A statement of GLP compliance was included.

Test Material: Acticide DCOIT, batch LM 437, white\yellow crystalline solid
Dosage: 0.5 mL

Species: Three male New Zealand White albino rabbits
Age: Not given
Weight: 1.54 kg to 1.94 kg before dosing
Source: Sarabhai Research Center, Baroda, Gujarat, India

Summary:

1. **Toxicity Category**: Unknown
2. **Classification**: Unacceptable

Procedure (Deviations From 81-5): The ages of the rabbits were not given in the study. This study does not indicate whether the test material was moistened. If the test substance was moistened, the lab report does not indicate the procedure used.

Results:

Rabbit	Erythema\edema after patch removal					
	1 hour	24 hours	48 hours	72 hours	Day 7	Day 14
1	3/2	3/2	3/2	4/4	4/3	4/3
2	3/2	2/2	2/2	2/2	2/2	4/4
3	3/2	2/2	2/2	1/2	½	4/3

DATA REVIEW FOR DERMAL SENSITIZATION TESTING (81-6, 870.2600)

Product Manager: Marshall Swindell
MRID No.: 47802215

Reviewer: Chris Jiang
Study Completion Date: Feb. 9, 2001
Report No.: 2870

Testing Laboratory: JAI Research Foundation
Author: Dr. T. Purshottam, Ph.D., M.Sc.

Quality Assurance (40 CFR 160.12): A statement of GLP compliance was included.

Test Material: Acticide DCOIT, batch LM 437, white\yellow crystalline solid
Positive Control: 2-Mercaptobenzothiazole

Species: Hartley guinea pig
Weight: ♂: 320 g to 442 g at induction, ♀: 274 g to 425 g at induction
Age: Not given
Source: Haffkine Biopharmaceutical Corporation Ltd., Mumbia, India

Method: Magnusson and Kligman Guinea Pig Maximization Test

Summary:

1. **This Product is a dermal sensitizer.**
2. **Classification**: Upgradable

Procedure (Deviation From §81-6): The ages of the animals were not given in the lab report. This deviation had no impact on the integrity of the study. This study does not indicate whether the test material was moistened. If the test substance was moistened, the lab report does not indicate the procedure used.

Procedure: After preliminary testing, the main study was undertaken.

Induction Phase – Intradermal Injections:

Day 0 – Treatment Group: Three pairs of intradermal injections of 0.1 mL volume were given in the scapular region which was cleared of hair so that each pair of injection was sited contralaterally to the median line of the animal. The injections were as follows: (1) Injection 1: a 1:1 mixture (v/v) Freund's Complete Adjuvant (FCA) with distilled water; (2) Injection 2: 5% Acticide DCOIT in propylene glycol; and (3) Injection 3: 5% Acticide DCOIT in propylene glycol formulated in a 1:1 mixture (v/v) FCA with distilled water. Injection 1 and Injection 2 were administered close to each other on the scapular region while Injection 3 was administered towards the caudal part of the test area.

Day 0 – Control Group: Propylene glycol was injected in the place of Acticide DCOIT to animals of the control group.

Skin reactions were observed at 24 hours after intradermal injections following the Magnusson and Kligman grading scale.

Induction Phase – Topical Application:

Day 6 – Treatment Group: As Acticide DCOIT was found to be irritant, 10% sodium lauryl sulfate in vaseline was not applied on flanks of all animals on Day 6.

Day 7 – Treatment Group: 0.2 mL of 25% Acticide DCOIT in 80% alcohol was applied to the left flank and was held in contact by an occlusive dressing for a period of 48 hours.

Day 7 – Control Group: 0.2 mL of 80% alcohol was applied to the left flank and was held in contact by an occlusive dressing for a period of 48 hours.

Skin reactions were observed at 24 hours after patch removal on Day 10, following the Magnusson and Kligman grading scale.

Challenge Phase – Topical Application:

Day 21 – Treatment and Control Groups: The flanks of treated and control animals were cleared of hair. The challenge dose of 0.2 mL of 5% Acticide DCOIT in acetone was applied to the right flank of all animals. The patches were held in contact by an occlusive dressing for a period of 24 hours.

Skin reactions were observed at 24 and 48 hours after patch removal and evaluated as per the Magnusson and Kligman grading scale.

Results:

Induction Phase:

Intradermal Injections (5% of test substance in propylene glycol): Mild erythema was observed in animals of the treatment group.

Topical Application (25% of test substance in 80% alcohol): Mild erythema was observed in animals of the treatment group.

Challenge Phase:

Topical Application – Test Animals (5% of test substance in acetone): 60% animals of the treatment group exhibited a positive skin response at 24 hours and 45% animals of the treatment group exhibited a positive skin response at 48 hours. The skin reaction score was "1" (discrete erythema) in 11 animals and "2" (moderate erythema) in 1 animal at 24 hours. The skin reaction score was "1" in 9 animals at 48 hours. The result shows that Acticide DCOIT is a moderate sensitizer (Grade III) to guinea pigs when using the guinea pig maximization test.

Topical Application – Control Animals: No erythema was observed in any of the control animals.

Twenty-four hours after challenge, 12/20 guinea pigs showed a sensitization response to the test material. Forty-eight hours after challenge, 9/20 exhibited a response to the test material.

The historical positive control showed appropriate results.